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Quantitative Determination of Swertiamarin in *Swertia chirayita* by HPTLC

LAIQA ANJUM, ZAINUL A. ANSARI, ARADHNA YADAV, MOHD. MUGHEES, JAVED AHMAD & ALTAF AHMAD*

Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi 110062, India

*Address for correspondence Email: ahmadaltaf@rediffmail.com

Abstract: A simple, rapid, and accurate High-Performance Thin-layer chromatographic (HPTLC) method has been developed for the determination of Swertiamarin in aerial parts of *Swertia chirayita* procured from Nepal & collected from Gangtok and Kumaon respectively. The methanolic extracts of *Swertia chirayita* samples were applied on TLC aluminum plate pre-coated with Silica gel 60 GF_{254} and developed using a solvent system containing Ethyl acetate : Methanol : Water (7.7 : 1.3 : 0.8, v/v/v) as a mobile phase. The densitometric detection of spots was carried out using a UV detector at 245 nm in absorbance mode. This system was found to give compact spot for swertiamarin (R_f value = 0.53±0.02). The calibration curve was found to be linear in the range of 200 to 1000 ng/spot. The limit of detection and quantification were found to be 50 and 200 ng/spot, respectively for swertiamarin. The highest and lowest concentration of swertiamarin in *Swertia chirayita* was found to be present in sample from Nepal and Gangtok, Sikkim respectively. The method was validated in accordance with ICH guidelines.

Key words: *Swertia chirayita*, secoiridoid glycoside, Swertiamarin, HPTLC, Gentianaceae.

Introduction

Swertia chirayita (Roxb.) H. Karst. is an important medicinal plant, belongs to the family Gentianaceae [1]. *Swertia chirayita* commonly known as Chirata/Chirayita is an erect annual or perennial herb, found in Himalaya and Meghalaya at an altitude of 1200-1300 m.The plant has been used in variety of health disorders, including asthma, colic, colon cancer, constipation, diarrhea, dyspepsia, liver ailments, nausea [3-4], constipation, diarrhea, dyspepsia, liver ailments, nausea [4-5], epilepsy, ulcer, melancholia and certain types of metal disorders [6]. This plant has also been reported to possess antidiabetic [7], anti-inflammatory [8] hepatoprotective [9], antibacterial [10], antioxidant [11] antimalarial, anthelmintics and antipyretic properties [12].

SWERTIAMARIN IS A SECOIRIDOID GLYCOSIDE [13-14] PRESENT IN MEMBERS OF GENTIANACEAE [15]. IT HAS CNS-DEPRESSANT EFFECT AND ANTICHOLINERGIC ACTIVITY [16-17]. IT IS A REPRESENTATIVE CONSTITUENT OF MANY CRUDE DRUGS MARKETED IN JAPAN AND OTHER COUNTRIES AND THESE CRUDE DRUGS ARE NORMALLY EVALUATED BY THEIR SWERTIAMARIN CONTENT [18]. ALTHOUGH HPTLC METHODS FOR THE ESTIMATION OF SWERTIAMARIN IN *SWERTIA CHIRATA (*WALL) CLARK, *E. LITTORALE* BLUME, AND MARKETED FORMULATIONS CONTAINING *ENICCOSTEMA LITTORALE* BLUME HAVE BEEN REPORTED [14, 19]. PRESENT STUDY DEALS WITH THE

QUANTITATIVE DETERMINATION OF SWERTIAMARIN IN *SWERTIA CHIRAYITA* (ROXB. EX FLEMING) H. KARST COLLECTED FROM THREE DIFFERENT PLACES.

MATERIALS & METHODS:

Plants of *Swertia chirayita* were collected from three different regions of Himalayas they were procured from Nepal, Gangtok, Sikkim and Kumaon, Uttarakhand respectively. These plant samples were identified by Prof. Javed Ahmad, Department of Botany, Jamia Hamdard. The plant material was cleaned and dried in the shade for a week at room temperature it was then powdered to 40 mesh and stored at 25 ºC.

Standard compound swertiamarin (99%) was purchased from Chromadex (Life Technologies, India). All the solvents and reagents used in the experiments were of analytical grade**.**

Preparation of Mobile Phase & Standard Solutions

Mobile phase was prepared by mixing Ethyl acetate: Methanol: Water in the ratio of 7.7:1.3:0.8 respectively. A stock solution of swertiamarin (1 mg/mL) was prepared by dissolving 2.0 mg of the standard swertiamarin accurately weighed in 2 mL methanol in a volumetric flask. Standard solution of 200 µg /mL was prepared from the stock solution by transferring 200 µL of stock solution, and diluting to volume with methanol (800 µL). Appropriate quantities of this standard solution were spotted to obtain swertiamarin in the range of 200-1000 ng/spot.

Preparation of Sample Solutions

Powdered samples of *S. chirayita* aerial parts (1g, accurately weighed) were extracted with methanol (2×25mL) for 24h at room temperature. The combined extracts were filtered through Whatman Filter paper No. 42. Extracts obtained were concentrated on rotary evaporator (R-200/205/V (Buchi) in vacuum to 10 mL.

Instrumentation and chromatographic conditions

HPTLC was performed on 20×10 mm aluminium backed plate coated with 0.2 mm layers of silica gel 60 F_{254} (E-Merck, Germany). Standard solutions of swertiamarin and the samples were applied to the plates as 5 mm wide band, 10 mm apart and 10 mm from the bottom and sides using a CAMAG Linomat V sample applicator fitted with a CAMAG Microlitre syringe (CAMAG, Germany). Linear ascending development of the plates to a distance of 80 mm was performed with Ethyl acetate: Methanol: Water

(7.7:1.3:0.8) v/v/v, as mobile phase in a 20×20 twin-trough glass chamber previously saturated with mobile phase vapor for 15 min at 25 ± 2ºC. The plate was dried completely and was scanned at 245nm using a CAMAG TLC scanner in absorbance mode, using the deutiriium lamp. The slit dimensions were 4 mm×0.1 mm and the scanning speed was 20 mm s^{-1}. Visualization of the spots was performed by spraying the plates with anisaldehyde reagent (anisaldehyde-glacial AcOH-MeOH-H_2SO_4 (0.5:10:85:5, v/v/v/v). After spraying the plates with the reagent, under 366-nm UV light this standard emits green light. A calibration curve in this study was plotted between the amount of analyte (swertiamarin) versus average response (peak area and regression equation was obtained Y= 8.349X+507.3 over the concentration range of 200-1000 ng/spot with respect to the peak area with a regression coefficient of 0.9961.

Method Validation

The developed method is validated as per the International Conference on Harmonization (ICH) guidelines Q_2 (R_1) [20]. By determining linearity, precision, accuracy, limits of detection (LOD), limits of quantification (LOQ) and recovery. Linearity of the method was evaluated by constructing calibration curves at nine concentration levels. Calibration curves were plotted over a concentration range of 200-1000 ng/spot. Aliquots of standard working solution of swertiamarin were applied onto the plate.

The calibration curves were developed by plotting peak area versus concentrations. The intraday and inter-day precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for chosen concentration of standard solution of swertiamarin for the proposed method. The results were reported in terms of relative standard deviation (% RSD). The accuracy of the method was determined by calculating recovery of swertiamarin by the standard addition method. Known amounts of standard solution of swertiamarin (50, 100 and 150 ng) were added to pre-quantified sample solution. Accuracy was expressed as percent recovery. The limit of detection (LOD) and Limit of quantification (LOQ) were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines [20].

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where σ= Standard deviation of the response; S=Slope of calibration curve

The specificity of the method was ascertained by analyzing standard compound and sample. The spot for swertiamarin in the sample was confirmed by comparing R_f and spectra of spot with that of standard. The peak purity of the swertiamarin was assessed by comparing the spectra at three different levels i.e. peak start, peak apex and peak end positions of the spot.

Standard solution of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 µL was applied to TLC plate in six replicates (n=6) to obtain final concentration range of 200-1000 ng/spot. The data of peak versus standard concentration was treated by linear–square regression samples chosen for the study were 200, 400 and 800 ng/spot.

RESULTS AND DISCUSSION

Initial trial experiments were conducted to select mobile phase for accurate analysis of the standards. Of the various mobile phases tried, mobile phase consisting of Ethyl acetate: Methanol: Water (7.7:1.3:0.8) v/v/v gave a sharp and well defined peak of swertiamarin at R_f value of 0.53 in standard (Figure 1) and samples respectively(Figure 2). Well defined spots were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature. For determination of linearity curves of area vs concentration, different amounts of stock solution of swertiamarin was applied for HPTLC plate analyses.

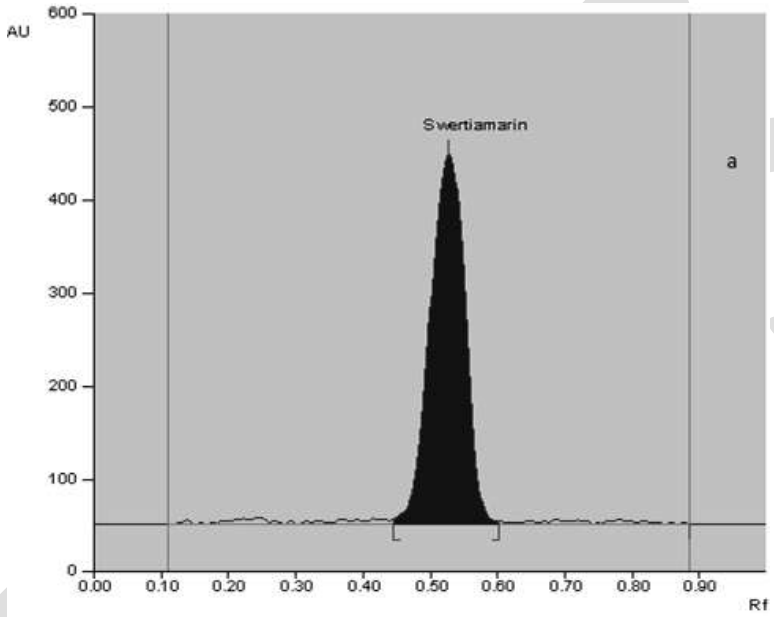

Figure 1: **TLC densitogram showing swertiamarin standard**

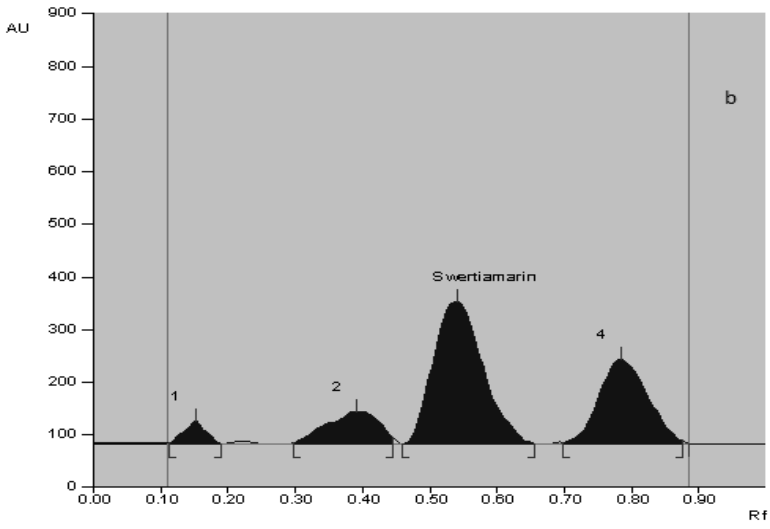

Figure 2: TLC densitogram resolution of swertiamarin in a sample

This method was validated for linearity, precision, specificity and accuracy. Calibration was linear in the concentration range of 200-1000 ng. The linear regression equation was found to be Y= 8.349X+507 for swertiamarin, while the correlation coefficient (r^2) was 0.9961 with high reproducibility and accuracy (Table 1). The proposed method, when used for estimation of swertiamarin after spiking with 50, 100 and 150% of additional standard afforded recovery ranging from 98.12-100.2% for swertiamarin was obtained as listed in (Table 2). The RSD of recovery of swertiamarin of was ranged from 0.13-0.39 (Table 2). The intra and inter day precision, as coefficient of variation (CV%) and accuracy of the assay determined at swertiamarin concentration of 200, 400 and 800 ng/spot has been summarized in (Table 3). The intra day precision (n=3) was≤1.35 The inter day precision over three different days was ≤2.19 The intra-day and inter day accuracy were in the range of 99.37-100.48 and 98.72-99.51 respectively.

The repeatability of the method was studied by assaying six samples of swertiamarin at same concentration under the same experimental conditions the values were within the acceptable range and so we concluded that the method was accurate, reliable and reproducible.

The robustness of the method was established by introducing small changes in mobile phase composition and chromatograms were run. The plates were prewashed by methanol and activated at 50±5ºC for 2,5,7 in prior to chromatography.

Limit of detection and limit of quantification were calculated by method as described in validation section and was found to be 50 ng and 200 ng/spot respectively, which indicates the ample sensitivity of the method.

The specificity of the proposed method was determined by comparing the sample and standard peak for its R_f and UV spectra. The peak purity of swertiamarin was assessed by comparing the spectra at three different levels i.e. Peak start, peak apex and peak end position.

A good resolved single spot of swertiamarin was observed at Rf value 0.53±0.02 in the chromatogram of the samples. the swertiamarin content in different samples of *Swertia chirayita* was observed and calculated (Table 4)

This HPTLC method has been developed for the determination of swertiamarin in *S. chirayita* collected from from different locations of Himalaya. The proposed method is simple, precise, specific accurate, less time consuming and cost effective. This method has been developed with high precision and economic considerations. Statistical analysis showed that the method is suitable for the analysis of swertiamarin. This method can be used for authentication of this plant and is also suitable for quality control and standardization of drugs derived from *S. chirayita.* It was found that plant sample collected from Dora, Himachal Pradesh contained highest amount of swertiamarin, so this region could be considered as a suitable region for the cultivation of *S. chirayita*.

Table 1: Linear Regression Data for Calibration Plot

Parameters	Observation for swertiamarin
Linearity range (ng/spot)	200-1000
Correlation coefficient (r^2)	0.9961
Regression equation	Y= 8.349X+507.3
Slope ±SD	8.349±0.156
Intercept ±SD	507.3 ± 0.968
LOD ng/spot	50
LOQ ng/spot	200

Table 2: Recovery Studies of Swertiamarin

Excess drug added to analyte	Theoretical content (ng)	% Recovery	% RSD
0	100	99.53	0.39
100	150	98.12	0.13
150	200	100.2	0.21
200	250	99.59	0.31

Table 3: Precision Study of Swertiamarin

Amount Applied ng/spot	Intraday Precision % RSD, n=3	Interday Precision % RSD, n=3
200	0.52	0.73
400	0.32	0.45
600	0.55	0.25

Table 4: Swertiamarin Content in Sample Extract (%w/w of sample) from different Locations

Compound	Place of collection	% w/w of sample mean ±SD
Swertiamarin	Nepal	1.42±0.006
Swertiamarin	Kumaon, Uttarakhand	0.859±0.027
Swertiamarin	Gangtok, Sikkim	0.704±0.009

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